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VAM OCCURENCE ON FOUR INDONESIA FORESTRY SPECIES

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ADANYA VAM PADA EMPAT SPECIES TEGAKAN HUTAN DI INDONESIA

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Abstract

Four forestry plantations in Wanagama I Forest Research Center, Yogyakarta were studied *(T. grandis, A. holosericea, A. mangium,* and *S. macrophylla).* Pot culture and direct collecting methods were apllied to study spore characteristics to detect the occurence of VAM. There were 16 different spore types described, including the common found genus, *Glomus*, some examples of *Sclerocystis, Scutellispora* and possibly *Acaulospo ra.* There were two types could not be categorized into any genus.

Key-words : vesicular-arbuscular mycorrhiza

Abstrak

Penelitian dilaksanakan pada empat species tegakan hutan *(T. grandis, A. holosericea, A. mangium,* and *S. macrophylla).* di Pusat Penelitian hutan Wanagama I, Yogyakarta. Metode budidaya pot dan koleksi langsung digunakan untuk meneliti jenis spora yang mencirikan adanya VAM. Terdapat 16 tipe spora termasuk di da lamnya genus yang mudah dijumpai *Glomus*, beberapa contoh genera *Sclerocystis, Scutellispora* dan kemung kinan *Acaulospora.* Didapatkan 2 tipe spora yang tidak dapat digolongkan ke dalam genus yang telah dikenal.

Kata Kunci : vesicular-arbuscular mycorrhiza

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Introduction

VA mycorrhizal fungi form the dominant type of myco rrhiza in the tropics (Mosse, 1981; Mikola, 1980) and have mostly been studied in relation to anatomy and morphology, host and symbiont interactions, physiology and nutrient uptake. One old study done in Java, Indonesia, found that most plant species within 56 observed families were mycorrhizal (Janse, 1897). However, Janse did not study the fungal species. Only a few studies in VAM done in the tropics have concerned fungal species identification such as a study on VAM occurence in Cuba (Herrera and Ferrer, 1980). They found 18 VAM spore types, of which 9 spore types were in *Glomus*, 2 types were comparable to *Gigaspora calospora* and *Gigaspora heterogama*, and one type resembled *Sclerocystis coremioides* Berk. & Broome. VAM fungi associated with cacao and oil palm in Malaysia were studied by Nadarajah (1980), and she found that *Glomus* was the dominant type and *Gigaspora*, *Acaulospora* and *Sclerocystis* were the minor types observed within the 22 observed spore types (pers. comm.). VAM fungi associated with woody species in the Phillipines (Reynaldo E. Dela Cruz, professor at Univ. of the Phillipinnes at Los Banos, personal comm.) found 20 types of endomycorrhiza. A study done in New Zealand (Hall, 1977) found 19 identified species with four new ones : *Glomus pallidus* Hall, *G. magnicaulis* Hall, *G. invermaius* Hall, *G. infrequens* Hall, and one new combination of *G. tenuis*. He found two *Sclerocystis* species: *S. rubiformis* Gerdemann & Trappe and *S. coremioides* Berk. & Broome; two *Gigaspora* : *G. aurigloba* Hall and *G. margarita* Becker & Hall; and three *Acaulospora* species : *Acaulospora laevis* Gerdemann & Trappe and two unidentified spores. New VAM fungal species have been found in tropical Colombia (Glomus glomerulatum Sieverding and Toro, Sieverding and Toro, 1987), *Acaulospora splendida* Sieverding was found in Costa Rica (Sieverding, 1988), and *Glomus callosum* Sieverding and *Acaulospora undulata* Sieverding were found in Africa (Sieverding, 1988).

The number of described species of VA mycorrhizal fungi has increased every year starting from the work of Gerdemann and Trappe (1974) which reported 31 species. In 1988, Morton reported that there were 126 VAM species which had been published in various papers. Recently, 150 described species were listed (Almeida, 1989).

VA mycorrhiza fungal classification underwent a revision proposed by Morton and Benny (1990). These fungi now belong to the Glomales in the Zygomycotina.

Species identification in VA mycorrhizal fungi is based on selected morphological characteristics of vegetative (hyphae, vesicles, and arbuscules) and reproductive (spores) stages (Morton, 1990). Therefore producing a large number of healthy spores is very useful to identify VA mycorrhizal fungi since characteristics of spore development can be obtained.

To differentiate among genera, stable characteristics such as spore wall structure (Walker, 1986a), presence of auxilliary cells;[1]germination shields[2]and bulbous suspensors,[3]are used as well as spore development in regard to the position relative to sporiferous saccule,[4] sporogenous hypha,[5] and fungal thallus (Walker, 1986b; Schenck and Perez, 1988; Morton, 1988; Berch, 1988).

More detailed stable characteristics are needed in identifying species within a genus. Spore wall structure (Schenck and Perez, 1988; Gerdemann and Trappe, 1974; Berch and Trappe, 1988 ; Berch, 1988; Morton, 1988) seems to be the key to species identification. This inludes : number of walls and wall groups, spore wall types, and wall ornamentation (Walker, 1986a ; Morton, 1988), as well as reaction of spore walls to certain mountants and Melzer's reagent (Morton, 1988).

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1. auxilliary cell : singled or clustered ornamented vesicles formed on spiraled hyphae in the soil by Gigaspora or Scutellispora species (Berch, 1988).
2. germination shield : wall and membrane bound cytoplasmic intrusion between spore wall layer groups that may be two- to many- lobed and that gives rise to germ tubes (adapted from Walker and Sanders 1986 in Berch, 1988).
3. bulbous suspensor : a hyphal swelling on which spores of *Gigaspora* and *Scutellispora* species are borne (synonymous : sporogenous cell) (Schenck and Perez, 1988).
4. sporiferous saccule : terminal (hyphal swelling forming before spore which then may be lateral (*Acaulospora* sp.) or intercalary (*Entrophospora* sp.) on the subtending hypha (Berch, 1988).
5. sporogenous hypha : (subtending hypha = sporophore) a spore-bearing structure, usually of brached or unbranched hyphae on which spores develop (Schenk and Perez, 1988).

The red reaction was observed for the innermost membranous wall of some species of *Glomus, Acaulospora,* and *Scutellispora;* the orange reaction was reported for *Glomus albidum* Walker & Rhodes and *Scutellispora fulgida* Koske & Walker; the purple reaction was observed in some species of *Acaulospora* (Morton, 1988).

Species identification based on available descriptions is difficult since many have mentioned that this relates to lack of uniformity in descriptions. Efforts to create a synoptic key for this fungus have been published (Gerdemann and Trappe, 1974), or worked on (Berch pers. comm.). A compilation of the Endogonaceae done by Berch (1988) proposed a formatted description to unify spore information which would be beneficial for taxonomical studies.

Being aware of only a few studies in which VA mycorrhizal fungal species have been identified from the tropics, especially Indonesia, the objective of this present study is to determine the occurence of VA mycorrhiza fungi associated with four forestry species : *Tectona grandis, Acacia holosericea, Acacia mangium,* and *Swietenia macrophylla.*

Materials and Methods

The sampling sites were four plantations *(T. grandis, A. holosericea, A. mangium,* and *S. macrophylla)* in Wanagama I Research Station, Yogyakarta, Indonesia. Since the root branching, length, and fine root abundance of each species is different, total root and soil samples for *A. mangium, A. holosericea, T. grandis and S. macrophylla* were 90, 54, 45 and 36 respectively. To identify mycorrhizal fungi, pot culture methods were applied (Ferguson and Woodhead, 1984) in order to produce enough spores.

Onion plants (*Allium* sp.) were germinated directly from 10 seeds in each pot. These experimental pots were placed in a growth chamber where the environment was set to artificial tropical conditions with 60% humidity, temperature 28^{O} - 30^{O} C, and 14 hours light per day with an average light source of 217.5 μ mol m-2 sec-1 (measured by Radio quantum meter, Li Cor type Li 185). After germinating, the plants were thinned to 3 plants in each pot. Plants were watered with distilled water every 2 days and rotated every week in order to reduce bias in physical conditions.

Plants were grown for 5 months, in addition, spores were collected directly from field-collected soils that had been air-dried. Spore collection was done using wet sieving and decanting method (Gerdemann and Nicolson, 1963 in Schenck, 1982) with series of 5 sieves from 1 mm up to 53 μ m openings. Spores were selected manually under the dissecting scope and slide preparation was carried out to group spores with similar morphological characteristics.

The spores obtained from the original soils were not healthy and a limited number of similar spores were found. Because of this, the descriptions were incomplete.

Results and Discussion

Results from pot culture studies did not produce enough spores for identification. This failure can be accounted for by various means. First, soils as inoculants for tropical VAM fungi are less effective than colonized roots inoculants due to low density of viable spores in the tropics (Ian Alexander, Aberdeen Univ., pers. comm.). Parasitism of spores in tropical soils, however, is common (Nadarajah, 1980). Second, spore germination is affected by environmental factors which, in Vancouver, may not be appropriate for tropical VAM fungi. Third, host plants should be well adapted to the environmental conditions, acceptable as host for the VAM species in the inoculant, grow rapidly and have no pathogens (Menge and Timmer, 1984). Ian Alexander and Lee Su See (pers. comm) have found that all these criteria are critical, but the use of an inappropriate host may have been most problematic in this case.

From the spore collection study using original soil samples, 79 slide preparations were selected in which there were 16 spore types based on spore wall structure and other spore characteristics, such as spore color (observed under transmitted light), size, and hyphal characteristics.

Only the unidentified spore types were described completely in this paper and presented with the picture.

3.a. Sclerocystis sp.

I. U type

In respect to spore wall structure, overall wall thickness, spore size, shape, and sporocarp size, this collection resembles Sclerocystis sinuosa Gerdemann & Bakshi. However the present collections lack a peridium and have spore walls that are thickest at the apex rather than the base as described in the original publication.

The present specimen is similar in appearance to a spore in slide #7 of Morton's slide set (Morton, 1989) identified there as *S. sinuosa*. However, they both differ from the original description, which is clearly stated as having a sinous network of hyphae as the peridium. Determining how similar the present specimens are to Morton's material would require comparison with a description of that material, but this does not seem to exist.

Compared to *Sclerocystis microcarpus* Iqbal & Bushra , the present specimens are similar in lacking a peridium and having wall thickening at the spore apex. However, spore shape and size differ.

Spore color and thickening of spore wall at the base are the main characters of *Sclerocystis rubiformis* Gerdemann & Trappe (Gerdemann & Trappe, 1974) that do not fit this specimen. However, spore size, lack of peridium, spore wall, and shape are similar to the present specimen.

Despite some similarities to *S. sinuosa, S. microcarpus* and *S. rubiformis,* the present specimens cannot be identified as any described species.

IIa. L / LU type

IIb. L/LU type

These two types above are differentiated by their measurements. Based on the spore wall structure, these two taxa (IIa and IIb) cannot be categorized into any species of *Sclerocystis* because of the presence of a thick laminate wall with distinctive innermost layer. If spore arrangement can no longer be used to separate *Glomus* and *Sclerocystis* (Berch, pers.comm.), then these taxa resemble *Glomus vesiculifer* (Thaxter) Gerdemann & Trappe (Gerdemann and Trappe, 1974) in spore wall structure but not size, shape, or presence of a peridium-like layer. When Gerdemann and

Trappe (1974) erected the new combination *Glomus vesiculifer* (Thaxter) Gerdemann & Trappe, they suggested that E. tjibodensis from Java was a later synonym (Gerdemann and Trappe, 1974), so it seems that similar material has been described from Java.

Cited description of Sclerocystis pachycaulis Wu & Chen (Schenck and Perez, 1988) was not clear in the spore wall structure, and therefore cannot be compared with the present collection. However, spore size and color are similar to the present species.

3.b. Sporocarpic Glomus sp.

III. L / L-U type
IV. L type

The two spore types above cannot be separated in terms of major characteristics of sporocarp and spores. Spore wall structure more or less differs, however these differences could be only the thickness of each wall or the interpretation of wall structure as a unit wall or innermost layer of laminate wall.

Cited description of *Glomus microcarpum* Tul. & Tul. suppl. Berch & Fortin resembles the present specimen in spore size, shape, and wall structure, but differs in wall thickness, sporocarp size, and nature of sporocarp of *G. microcarpum* which has a peridium. However, description of the same species by Gerdemann & Trappe (1974) suggests that this species sometimes forms aggregations or small clusters not enclosed in a peridium.

Compared to *Glomus pallidum* Hall (Hall, 1977), spore shape, approximate spore size, wall structure and thickness, hyphal wall thickening and lack of a peridium are similar. Differences include subtending hyphae of *G. pallidum* which are bigger, have a closed pore and lack lateral swellings. The description of *Glomus aggregatum* Schenck & Smith emend. Koske (Koske, 1985) indicates similarities to the present specimen in the following characteristics : spore wall structure, thickness, shape and color; hyphal diameter, wall thickness, open pore, straight position; and lack of a peridium on the sporocarp. But comparatively bigger spores and sporocarps, and curved, infundibuliform or constricted subtending hyphae are present in *G. aggregatum*. Small dissimilarities could happen in descrip-

tions since the apparent characteristics of species depend on the spore condition and the number of observed spores. *G. aggregatum, G. microcarpum, G. pallidum* are the most similar species to the present specimen, even though none of these are exactly the same. This specimen needs verification using healthier spores.

V. U type

Glomus microaggregatum Koske, Gemma & Olexia most resembles the present specimens in spore shape, color, size, wall structure, and hyphal characteristics including hyphal diameter, pore, position relative to spores, wall thickness and thickening at the spore base. However, the present specimens form sporocarps and laterally swelling hyphae which were not described in *G. microaggregatum.*
Compared to the description of *Glomus pulvinatum* (P. Henn.) Trappe & Gerdemann, the present specimens are similar in spore color, spore wall thickness and probably structure; but not spore size and the presence of a peridium which differ from the present specimens.
Glomus microaggregatum and *G. pulvinatum,* are close to the present description, however, it cannot be identified as either of these two species, because of basic dissimilarities.

3.c. Nonsporocarpic Glomus sp.
VIa. E-L-Flexible Type

Compared to *Glomus ambisporum* Smith & Schenck (Smith & Schenck, 1985), spore wall structure, shape, size and lack of sporocarp are similar to this present specimen. Subtending hyphal characteristics, such as bigger diameter and occluded pore, are different.

Description of *Glomus ambisporum* Smith and Schenck (Smith and Schenck, 1985) has similarities to this present specimen in spore size, shape, hyphal diameter, and one possibility of the spore wall structure. In the description, it says that *G. ambisporum* can form in sporocarps or singly in soil, and spores of the second morph have a single group of three spore walls. These are evanescent, laminate, and membraneous respectively from outermost. The evanescent wall in old spores sometimes cannot be detected anymore since it is gradually degraded as spore mature. Based on these

consideration, the spore wall structure is similar to the present specimen. However, since only one spore was observed, we cannot compared it to the second spore morph which forms dark brown sporocarp.

Compared to *Glomus diaphanum* Morton and Walker, spore size, shape, wall structure, lack of sporocarp, hyphal diameter and hyphal wall thickness resemble the present specimen. Tendency to form cluster of spores and consistent hyphal wall thickness in *G. diaphanum* differ.

VIb. E-L-Flexible Type

Compared to description of *Glomus diaphanum* Morton and Walker, the following characteristics are similar to the present specimens : lack of sporocarp, spore size, shape, spore wall structure and thickness, hyphal diameter and hyphal wall. But the extention of the second wall into the subtending hypha was not observed and spore color in the present specimen is darker. Decreasing of hyphal wall thickness from the point of attachment is not comparable.

In respect to spore wall structure, hyphal diameter and hyphal wall at the point of attachment, description of *Gl.cerebriforme* McGee closely resembles present specimens. The tendency to form sporocarps, smaller spore size, white spores, and hyphal wall decreasing within 50 µm from attachment in *G. cerebriforme* are different.

The second spore type in *Glomus ambisporum* Smith and Schenck (1985) was described having a broad range of spore size, globose to subglobose shape, single wall group, 5 - 10 µm hyphal diameter. These characteristics largely resemble the present specimens, except for the presence of an evanescent outermost wall and hyaline spores in *G. ambisporum*.

VII. OT 21 type.

A few characteristics in original description of *Glomus aggregatum* Koske (Koske, 1985), spore size, color, spore wall structure, and hyphal characteristics including diameter, wall thickness, position, pore, and constricted hypha are the most similar to present specimen. However, the present specimens have thicker spore wall, form in a loose aggregate, and vesicle like structures as big as the smallest spore are observed.

This present specimens resemble *Glomus deserticola* Trappe & Bloss in spore shape, wall structure, and hyphal diameter, but differs in spore color, spore wall thickness and the presence of vesicle like structures.

VIIIa. L + U type These three different types were based on the

VIIIb. L + U type measurement of minor spore characteristics

VIIIc. L + U type

Compared to *Glomus delhiense* Mukerji, Bhattacharjee & Tewari,, spore wall structure, spore color, and subtending hyphae are similar but spore size is bigger in *G. delhiense.*

IX. E-L-U Type

Description of *Glomus etunicatum,* Becker & Gerdemann resembles the present specimen in spore wall structure but not in spore wall thickness and spore size.

Xa. L type or E + L type

Compared to description of *Glomus monosporum* Gerdemann and Trappe (Gerdemann and Trappe, 1974), spore shape, size, spore wall structure and hyphal diameter are similar to the present specimen. Tendency to form sporocarp, the presence of peridium, double hyphae and curved hypha in *G. monosporum* are different from these specimens.

Xb. OAm10 type.

Description of *Glomus multisubtensum* Mukerji, Bhattacharjee & Tewari spore size, shape, color, and phenomena of having more than one hypha resembles this present specimens. However, *G. multisubstensum* has two separable walls and the spores tend to make aggregates of 5 to 8 spores.

XI. E-L-M Type

The description of *Glomus geosporum* (Nicol. & Gerd.) Walker (Walker, 1982) is much like the present specimen in spore shape, color, size, wall structure, lack of sporocarp, and characteristics of subtending hypha including hyphal diameter and length. However, hyphal constriction, hyphal wall thickening at the spore base, and the spore content which become crystallized in the present collection differ from *G. geosporum*.

XII. TO6 Type

Description of *Glomus claroideum* Schenck and Smith, resembles the present specimen in spore shape, size, color, wall structure and the subtending hypha characteristics. Both do not form sporocarps as well. In the present specimen the subtending hyphal wall thickness decreasing within 25 µm from point of attachment which was not described in *Glomus claroideum*.
Compared to *Glomus diaphanum* Morton & Walker (Morton and Walker, 1984) the present specimen has bigger spore size, and bigger hyphal diameter.

3.d. Scutellispora sp.

XIII. U-2M Type.

XIV. U-M Type

With in *Gigaspora* the available descriptions cited by Schenck and Perez (1988) seem not to agree with ours since both present specimens have two clearly separated wall groups rather than a single group. Germination shields might not have developed yet. The presence of membraneous walls in the inner group of both specimens is similar to *Scutellispora* spore wall structure. Comparison to species in *Scutellispora* cannot be carried out, because mature spores detected by the presence a germination shield would be the appropriate specimens to compare to available descriptions.

3.e. Unidentified genera

XV. U-L/Flexible Type (Acaulospora sp.?)

Sporocarps unknown. Spores globose, yellow, 120 - 125 x 120 - 125 µ m. Spores borne on single subtending hypha, yellow, straight, open pore, and relatively small (4-5 µm) compare to the spore size. Spore wall structure : one group with two or more walls : wall 1, possibly unit, brownish yellow, rough surface, 1 - 2 µ m, wall 2, laminate or some flexible layers, hyaline, smooth with many sublayers. See figure 1.

When spore was broken, there was a tendency of walls 1 and 2 to fold into a radiate pattern which may be because of the consistency of the wall. The spore contents are very crystalline, thick and tend to stick to the spore wall (PVA as a mountant). The nature of the spore contents is different from common spore contents which is usually thick and oily.

This type is separated from the previous type because of the spore wall characteristics, the way the wall folds when spore was broken, and the distinctive spore contents, even though the subtending hypha attachment in the slide is not clear enough to differentiate between *Glomus* and *Acaulospora.*

Compared to the slide prepared by Morton (1989), even though the appearance is similar to *A. laevigatum* nom.ined. in the way the wall is folded and spore contents, the spore wall structure in *A. laevigatum* appears more complex than this spore type and the original description is not available. The spore collected from the field may be old and, when the spore was broken, the flexible / membranous / or coriaceous layers stuck together in such a way to look like a laminate wall.

XVI. U-M-C Type (Acaulospora sp ?)

Sporocarp : unknown. Spores : globose, yellow (10I1g Y), 140 x 140 µ m (one spore only). Subtending hypha not observed, only scar of hyphal attachment, brownish yellow, 16 x 16 µ m, opening 2 fm. Germination shield : hyaline, approximately 68 x 68 µ m (boundary is not clear), formed between spore wall groups B and C.

Spore wall structure : consists of three groups:

Group A : wall 1, unit or two unseparated unit walls (1 and 2), yellow, 2.25 µ m, outer surface ornamented with rounded warts, brownish color,

closely packed (12 - 15 warts/10 μ m), each wart less than 1 fm in diameter.
Group B : walls 2 and 3, hyaline, membraneous (?), 1 μ m each.
Group C : walls 4, 5 and 6, with the outermost wall (4) coriaceous (?), hyaline, 2 μ m with patchy roughening of surface; middle wall 5 and the innermost wall 6, membraneous or coriaceous , hyaline, 2 and 1 μ m respectively. See figure 2.

The presence of a germination shield in this spore first suggested the genus *Scutellispora* even though the bulbous suspensor was not observed. There are four described *Scutellispora* species with three separable wall groups : *S. pellucida* (Nicolson & Schenck) Walker & Sanders (Koske and Walker, 1986) *S. savannicola* (Herrera & Ferrer) Walker & Sanders (cited from Schenck and Perez, 1982), *S weresubiae* Koske & Walker (Koske and Walker, 1986), and *S. tricalypta* (Herrera & Ferrer) Walker & Sanders (cited from Schenck and Perez, 1982). The specimen cannot be compared to *S. tricalypta* because structure and thickness of wall layers in every wall group, size and the color of the spore are different. Similarly, *S. weresubiae* differs except for spore size and the appearance of laminate wall type at the second layer. Comparing to *S. pellucida* does not work because of the appearance of laminate type in the second layer and unit type in the innermost of wall group B. The closest description to this spore type is *S. savannicola* except for the ornamentation on the outer layer and the fourth layer found in the specimen.

The evidence of having ornamentation on the outer layer and the presence of a delicate germination shield between wall group A and B can also suggest that this specimen belongs to genus *Acaulospora.*

Conclusions

From original soil of plantation and nursery, 16 different spore types were described based primarilly on spore wall structure. From these grouped spore types, *Glomus* is the most common genus, with some examples of *Sclerocystis, Scutellispora* and possibly *Acaulospora*. There was also a type that could not be categorized into any genus.

Our inability to identify any collection to species was based on the poor condition of the spores collected and the small number of spores. Another factor may be that the VAM fungi of Java are virtually unknown, and the fungi we saw may have been unknown to science.

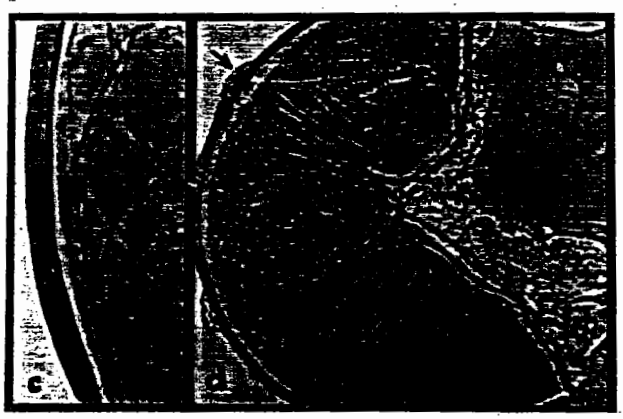

Figure 1 : c) Spore wall in other spore shows unit and membraneous (1 and 2) (bar = 10 μm) ; d) Hyphal attachment (arrow) (bar = 12.5 μm)

Figure 2 : b) Crushed spore, arrow pointing at scar (bar = 8 μm); c) Spore wall structure with 6 layers (1 to 6) separated into three groups (a, b, and c), arrows show ornamentation (at wall 1) and roughened layer (at wall 4) (bar = 8 μm).

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